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REMARKS

Claims 1, 5, 6, 9-11 and 14-27 are now pending. By this Amendment, claim 1 is amended and claims 27 is added.

In the Final Rejection mailed February 17, 2004, claims 1, 5, 6, 10, 11, 14, 19 and 20-26 were rejected under 35 U.S.C. §112, first paragraph, for allegedly lacking enablement. Applicants traversed this rejection, as well as the other rejections set forth in the final rejection, in the Amendment After Final Rejection that was filed on June 17, 2004. In further response to this rejection, the following comments are included:

Based on filing of a Request for Continued Examination filed herewith, the Declaration submitted with the Amendment After Final Rejection on June 17, 2004 should now be fully considered. It is respectfully submitted that this Declaration clearly demonstrates that antisense technology is enabled at least with respect to treating metastatic tumor cells of epithelial tissue origin. Although the evidence that has been provided relates to breast cancer and melanoma, it is respectfully submitted that these experiments are representative of other types of metastatic tumor cells of epithelial tissue origin. Thus, it is respectfully submitted that the full scope of claim 1 is enabled by the present specification.

As further evidence of this, attached are a number of scientific abstracts showing that thrombin receptors are expressed in a large number of cancers (prostate, pancreatic, ovarian) and, furthermore, that expression and activation of this receptor induces cell proliferation in these cancers (Greenberg et al. 2003). These scientific articles show that the inventors have found a common motif demonstrated in epithelial cancers - - functional thrombin receptor expression and activity, and that the inventors further found that closing this expression by administration of antisense sequences, as shown for two members of this family (melanoma and breast cancer cells), would avoid further proliferation of cancer cells. Thus, it is

respectfully submitted that the present application clearly enables the treatment of metastatic tumor cells of epithelial tissue origin with antisense technology.

In view of the foregoing, it is respectfully submitted that this application is in condition for allowance. Favorable reconsideration and prompt allowance of claims 1, 5, 6, 9-11 and 14-27 are earnestly solicited.

Should the Examiner believe that anything further would be desirable in order to place this application in even better condition for allowance, the Examiner is invited to contact the undersigned at the telephone number set forth below.

Respectfully submitted,

James A. Oliff
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JAO:MLM/brc

Attachments:
4 Abstracts

Date: July 19, 2004

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1: Pancreas. 1998 Mar;16(2):189-94.

Characterization of functional thrombin receptors in human pancreatic tumor cells (MIA PACA-2).

Rudroff C, Schaffberg FI, Nowak G, Weinel R, Scheele J, Kaufmann R.

Department of Surgery, Friedrich Schiller University Jena, Germany.

In this article, the "tethered ligand" thrombin receptor was identified on human pancreatic tumor cells, MIA PaCa-2, using immunofluorescence studies with a monoclonal anti-thrombin receptor antibody. Pharmacological characterization, using 3H-labeled thrombin receptor activating peptide-6 (TRAP-6) as radioligand, demonstrated a single class of high-affinity binding sites (KD = 9.1+/-1.8 x 10(-7) M) and a binding capacity of 13.9+/-0.7 [mol/mg protein. These binding sites represent functional thrombin receptors, as shown by alpha-thrombin- and TRAP-6-induced mobilization of free intracellular calcium, protein kinase C translocation from cytosol to the cell membrane, and stimulation of DNA synthesis in MIA PaCa-2 cells. These results provide the first identification of tethered ligand thrombin receptor in human pancreatic cancer cells and suggest thrombin receptor involvement in mechanisms of human pancreatic tumor progression.

PMID: 9510143 [PubMed - indexed for MEDLINE]

ELSEVIER

A FUNCTIONAL THROMBIN RECEPTOR (PAR1) IS EXPRESSED ON BONE-DERIVED PROSTATE CANCER CELL LINES

CHRISTOPHER H. CHAY, CARLTON R. COOPER, JAMES D. GENDERNALIK, SARAVANA M. DHANASEKARAN, ARUL M. CHINNAIYAN, MARK A. RUBIN, ALVIN H. SCHMAIER, AND KENNETH J. PIENTA

ABSTRACT

Objectives. To identify genes important in prostate cancer metastatic to bone. Bone-specific metastasis is a common feature of prostate cancer and a significant cause of morbidity.

Methods. To identify factors involved in organ-specific metastasis, we used cDNA microarray analysis to compare a bone-derived cell line, VCaP, with a soft tissue-derived cell line, DuCaP. Both cell lines were derived from the same patient and spontaneously passaged.

Results. Forty-five genes were differentially expressed, and only seven of these also had increased expression in VCaP compared with normal prostatic tissue. Of these, protease-activated receptor 1 (PAR1) was verified as having increased expression by reverse transcriptase-polymerase chain reaction and Northern blot analysis, as well as by immunohistochemistry. PAR1 expression in a panel of prostate cancer cell lines demonstrated increased expression in those cell lines derived from bone metastases. Alpha-thrombin stimulation of the VCaP cells produced a dose-dependent mobilization of intracellular calcium compared with DuCaP, suggesting that PAR1 expressed on the VCaP prostate cancer cell line is functional.

Conclusions. These data indicate that a functional PAR1 is expressed on prostate cancer cell lines. The prostate cancer cell lines expressing PAR1 appear to have an association with increased bone metastases. UROLOGY 60: 760–765, 2002. © 2002, Elsevier Science Inc.

Prostate cancer metastasis to bone not only occurs early in the course of disease, but is the most common site of metastasis.[1,2] Clinical patterns of metastasis have been recognized for many years, but the interactions between prostate cancer cells and the bone marrow that allow for this specific and frequent finding remain poorly understood. Microarray analysis has recently been used to compare prostate cancer from patients and cell lines with normal prostatic tissue.[3,4] Although cDNA microarrays can be used to characterize cell lines and patient samples, no studies have addressed the differences in expression patterns important in determining organ-specific metastasis.

It has been increasingly recognized that hemostatic proteins and mechanisms associated with vascular biology are associated with cancer metastasis.[5] Although cancer cells can be associated with hypercoagulability, evidence is growing that hemostatic factors, including thrombin, may increase metastasis through adhesion and invasion. The thrombin receptor or protease-activated receptor 1 (PAR1) has been identified on platelets, endothelial cells, smooth muscle cells, and fibroblasts. Recent studies have identified functional thrombin receptors on cancer cells as well. Thrombin signal-

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A. H. Schmaier holds stock in, and is President of, Thromgen, Inc.

From the Division of Hematology/Oncology, Department of Internal Medicine, Department of Pathology, and Division of Urology, Department of Surgery, University of Michigan Medical School; and Thromgen, Inc., Ann Arbor, Michigan

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Internat J Cancer. (IJC)

Differential expression of *Protease Activated Receptor 1 (Par1)* and pY397FAK in benign and malignant human ovarian tissue samples

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Departments of Oncology[1], Obstetrics & Gynecology[2] Pathology[3], Hadassah-Hebrew University Hospital[1], POB 12000 and Shaare-Zedek Medical Center[2] Jerusalem 91120, Israel

Short title: *hPar1* expression in ovarian malignancies

Key words: PARs, thrombin, human ovarian tissues, proteases, extracellular matrix, carcinoma

Abbreviations used: PAR1; *Protease Activated Receptor 1*, FAK; focal adhesion kinase, FACs; focal adhesion contacts

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Abstract

Protease Activated Receptors (PARs) form a family of G-protein coupled receptors (GPCR) encoding their own ligands and uniquely activated via proteolytic cleavage. While proteases in general, have been implicated in the remodeling of the extracellular tumor microenvironment, the role of cell surface receptors activated by proteolysis is now emerging. In the present study we investigated the expression pattern of *hPar1* in ovarian carcinoma tissue samples. Abundant *hPar1* mRNA and protein were detected in "low malignant potential" and in invasive carcinomas, regardless of the histological subtype. In contrast, no *hPar1* expression was detected on the cell surface of normal ovarian epithelium. The differential expression pattern of *hPar1* was shown by *in situ* hybridization, immunohistochemistry and semi-quantitative RT-PCR analyses. In early stages of ovarian carcinoma (Ia), the contra lateral normal ovary showed strong PAR1 expression as opposed to the lack of expression in the ovarian epithelium obtained from normal individuals. In parallel, we analyzed the expression pattern of αvβ5 integrin and of activated focal adhesion kinase (FAK), a major focal contact protein, in these tissues. While abundant expression of αvβ5 integrin was observed in all tissues specimens examined, regardless of either normal or malignant, the level of activated FAK was differentially expressed. Phosphorylated FAK was seen in invasive ovarian carcinoma, but not in the normal ovarian epithelium. The abundant *hPar1* levels in pathological malignant ovarian carcinoma is likely to transmit signals leading to the phosphorylation of FAK and thereby alterations in the integrin functional state. Altogether our data suggest that *hPar1* and FAK cooperate to promote ovarian cancer malignancy.

Biochemistry 2003, 42, 702−709

Protease-Activated Receptor Mediated RhoA Signaling and Cytoskeletal Reorganization in LNCaP Cells†

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ABSTRACT: Thrombin and trypsin induce cell signaling through a subclass of G-protein-coupled receptors called the protease-activated receptors (PARs). In many cells, PAR signaling results in the activation of RhoA and other members of the Rho family of small GTPases which are involved in cytoskeletal reorganization. The expression of PARs and their role in the activation of Rho GTPases in prostate cancer cells are not clearly known. FACS analysis demonstrated that the androgen-dependent LNCaP cells express PAR1, PAR2, and PAR4 but not PAR3. Stimulation with thrombin and trypsin resulted in the rapid activation of RhoA in a dose-dependent manner with an EC_{50} of 1.0 and 5 nM, respectively. Activation of RhoA was enhanced by, but not dependent on, the presence of 1 nM dihydrotestosterone. Inhibition of the proteolytic properties of thrombin by hirudin and trypsin by diisopropyl fluorophosphate abolished the observed RhoA activation. Stimulation with 150 μM PAR-activating peptides TFFLRN (PAR1), SLIGKV (PAR2), and AYPGKF (PAR4) demonstrated that PAR1 and PAR2 mediated protease-activated RhoA signaling. Fluorescent microscopy studies showed that LNCaP cells treated with either thrombin (10 nM) or trypsin (10 nM) developed an increased number of filopodia, stress fibers, and focal adhesions relative to untreated cells. These observations represent the first report of PAR signaling in prostate cancer cells as well as the ability of PAR2 to mediate RhoA activation. Since the activation of RhoA is important for cytoskeletal reorganization, we postulate that PAR-mediated RhoA activation may be a major signaling pathway in the biology of prostate cancer.

Prostate cancer is the most commonly diagnosed malignancy after skin cancer and the second leading cause of cancer mortality in men in the United States (*1*). As a paracrine gland the prostate primarily functions by secreting a nutrient-rich fluid which comprises about 30% of the total seminal fluid (*2*). Prostatic fluid contains prostate-specific antigen (PSA),[1] a hormonally regulated serine protease that serves as a valuable tumor marker for prostate cancer (*2*). Several prostatic serine proteases, including PSA, are produced by the glandular epithelial cells and secreted into the prostatic fluid. The glandular epithelial cells also express transmembrane serine proteases, including TMPRSS2 (*3*) and hepsin (*4*−*6*). Prostatic serine proteases may cleave specific cellular substrates that influence cell growth and the progression or metastatic prostate cancer (*7*). These cellular substrates have been postulated to include growth factor precursors, regulatory factors of signal transduction and gene transcription, and cell surface protease-activated receptors. In this paper we show that intracellular signaling and cytoskeletal reorganization in the prostate cancer cell line LNCaP can be mediated by the proteolytically active serine proteases, thrombin and trypsin.

A major mechanism by which cellular behavior is influenced by serine proteases is through the activation of the protease-activated receptors (PARs), a subfamily of the G-protein-coupled receptors (GPCRs) (*8*, *9*). PARs are found in a variety of tissues and cells including platelets, endothelial cells, the gastrointestinal tract, the central nervous system, and the lungs. Numerous established cell lines derived from malignant tissues such as breast, colon, lung, and prostate contain mRNA for at least one and usually two or more of the four PARs (*8*). Gene knockout of PAR1 in mice shows normal development in half of the fetuses while the other

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[1] Abbreviations: PAR, protease-activated receptor; GPCRs, G-protein-coupled receptors; AP, activating peptide; AP-1, TFFLRN (PAR1); AP-2, SLIGKV (PAR2); AP-3, TFRGAP (PAR3); AP-4, AYPGKF (PAR4); FACS, fluorescent-activated cell sorter; GST, glutathione *S*-transferase; RBD, rhotekin binding domain; GST-RBD, glutathione *S*-transferase−RhoA binding domain fusion protein; EC_{50}, effective concentration for 50% response; DFP, diisopropyl fluorophosphate; DHT, dihydrotestosterone; PSA, prostate-specific antigen; PBS, phosphate-buffered saline; DAPI, 4′,6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; HRP, horseradish peroxidase; ECL, enhanced chemiluminescence; FBS, fetal bovine serum; GSH, glutathione; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TBS, Tris-buffered saline; AR, androgen receptor; GEF, guanine exchange factor; RGS, regulation of G-protein signaling; RT-PCR, reverse transcription polymerase chain reaction; RXR, retinoic acid receptor; PIP5 kinase, phosphatidylinositol 4-phosphate 5-kinase,

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